Microdialysis of the Interstitial Water Space in Human Skin In Vivo: Quantitative Measurement of Cutaneous Glucose Concentrations

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The purpose of this study was to evaluate the usefulness of a microdialysis technique for measurement of substances in the interstitial water space in intact human skin. Glucose was selected to validate the method. The cutaneous glucose concentration was measured by microdialysis and compared to that in venous blood. Single dialysis fibers (length 20 mm, 2,000 Da molecular weight cutoff) were glued to nylon tubings and inserted in forearm skin by means of a fine needle. Dialysis fibers were inserted in duplicate. Seven subjects were investigated after an overnight fast. Intradermal position of the dialysis probes was established by C-mode ultrasound scanning. The implantation trauma lasted 90–135 min as measured by laser Doppler flowmetry. Each dialysis fiber was calibrated in vivo by perfusing it with four to five different glucose concentrations. The perfusion rate was 3 μl/min. Regression analysis of the calibration curves yielded the relative in vivo recovery of glucose. The skin glucose concentration was calculated as that particular perfusate glucose concentration that resulted in no net glucose transport across the dialysis membrane. Correlation coefficient of the regression lines was 0.93 ± 0.03 (mean ± SEM). After the injection trauma had vanished, recovery was 20.5 ± 0.7%. Coefficient of variation (CV) on recovery was 10.9%. The cutaneous glucose concentration was 99.1 ± 1.8% of the glucose concentration in venous plasma water (CV 4.1%). These findings suggest that the microdialysis technique accurately and precisely can reflect biochemical events in the interstitial water space in human skin in vivo. *J Invest Dermatol 99:357–360, 1992*

Measurements pertaining to the interstitial water space in skin have generally been limited to analysis of samples from suction blisters. However, a method allowing entry to biochemical processes in intact skin would be preferable. Also, the samples should be caught at the site of formation, thus avoiding dilution due to diffusion and enzymatic degradation of the substances. Microdialysis might prove to be such a technique.

Microdialysis has been used for years for the assessment of in situ metabolic events at the extracellular level in animal brain preparations [1,2]. The microdialysis method has only lately been used in peripheral tissues, including subcutaneous adipose tissue [3,4] and myocardium [5]. So far, only preliminary results of skin microdialysis have been published [6].

The aim of the present study was to elucidate whether the microdialysis method is suitable for measurement of metabolic events in human skin in vivo and to measure the steady-state glucose concentration in skin after an overnight fast. Glucose was selected to validate the method because glucose equilibrates rapidly in its distribution volume. Therefore, comparisons can be made between glucose concentrations in the tissue and venous blood as long as strict steady-state conditions are present.

Manuscript received January 22, 1992; accepted for publication April 8, 1992.

This work was supported by the Medical Faculty, University of Copenhagen, King Christian X Foundation, Aage Bang's Foundation, and Novo's Foundation Committee.

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Abbreviations:

CV: coefficient of variation, (SD/mean) × 100

MATERIALS AND METHODS

Subjects Seven subjects, one woman and six men, participated in the study. One subject was studied on two occasions (see below). Mean age was 41 years (range 30–60 years). Four of the subjects were healthy volunteers and three were patients suffering from localized skin diseases. All experiments were performed on normal-appearing skin. None of the patients took any medication known to interfere with carbohydrate metabolism, and none had diabetes mellitus. All subjects gave informed consent, and the study was approved by the regional Ethical Committee (KF V 100.2050/91).

Skin Microdialysis Technique The probes, which are similar in principle to a microdialysis probe previously described for subcutaneous adipose tissue [3], were constructed by using a single dialysis fiber (Gambro GFE 18, Gambro Dialysaten AG, FRG) glued to gas-tight nylon tubings with cyanoacrylate and sterilized. The fibers (216 μm outer diameter; 8 μm wall thickness; 2,000 Da molecular weight cutoff) have a length of 20 mm and the outlet of the nylon tubing had a standardized length of 30 mm.

The inlet of the nylon tubing was connected to a microinjection pump (Carnegie CMA/100, Carnegie Medicin AB, Sweden) and perfused with isotonic saline at a rate of 3 μl/min.

In Vitro Experiments The relative recovery (dialysate/medium exchange ratio) of glucose depends on the perfusion rate. Therefore, the probe was placed in a vial containing a known glucose concentration. By varying the perfusion rate from 1 to 15 μl/min, relative recovery was estimated. The results are presented in Fig 1*A*. In the

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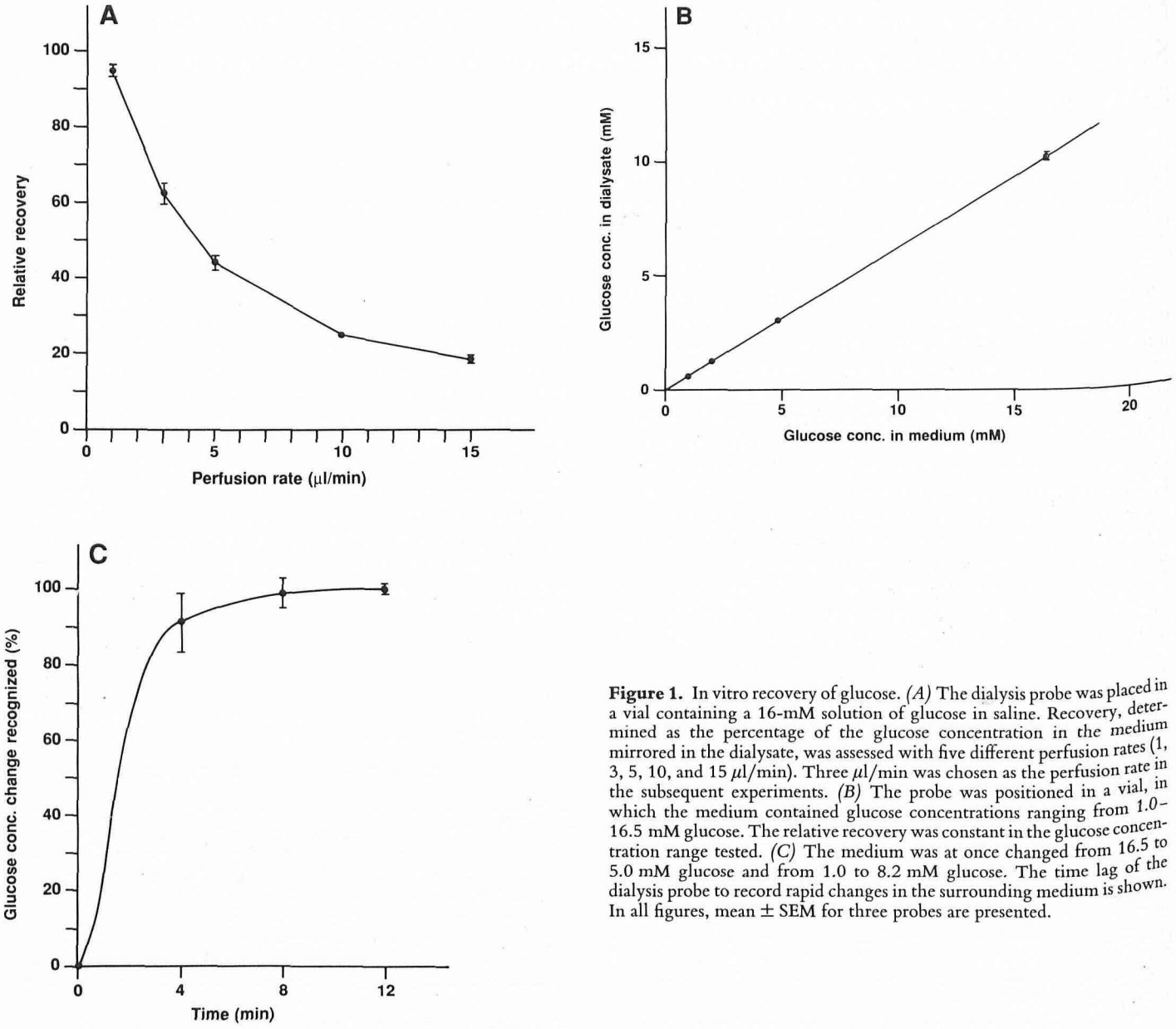

Figure 1. In vitro recovery of glucose. *(A)* The dialysis probe was placed in a vial containing a 16-mM solution of glucose in saline. Recovery, determined as the percentage of the glucose concentration in the medium mirrored in the dialysate, was assessed with five different perfusion rates (1, 3, 5, 10, and 15 μl/min). Three μl/min was chosen as the perfusion rate in the subsequent experiments. *(B)* The probe was positioned in a vial, in which the medium contained glucose concentrations ranging from 1.0–16.5 mM glucose. The relative recovery was constant in the glucose concentration range tested. *(C)* The medium was at once changed from 16.5 to 5.0 mM glucose and from 1.0 to 8.2 mM glucose. The time lag of the dialysis probe to record rapid changes in the surrounding medium is shown. In all figures, mean ± SEM for three probes are presented.

subsequent in vitro studies and in all in vivo experiments, the perfusion rate was 3 μl/min.

To characterize the dialysis fiber, the probe was positioned in a saline solution with different concentrations of glucose. The relative recovery of glucose in the dialysate was constant (60.5 ± 0.3%, mean ± SEM) in the range from 1.0 to 16.5 mM glucose. The results are presented in Fig 1*B*.

The sensitivity of the dialysis probe to record rapid changes in the surrounding medium was studied by switching the probe from 16.5 to 5.0 mM glucose and from 1.0 to 8.2 mM glucose. When 4-min samples were collected, 87% of the change in glucose concentration was recorded in the dialysate after 4 min, 98% was recognized after 8 min, and 100% was obtained after 12 min. The results are shown in Fig 1*C*.

Due to the low perfusion rate, and, with the in vivo experiments, the air-exposed inlet and outlet connections, evaporation during the sampling procedure might result in an overestimated glucose concentration in the dialysate. By using identical glucose concentration in the perfusate and in the medium and air-exposed perfused dialysis fibers, evaporation was estimated. It was shown that evaporation during the sampling situation per se was 3.7%. In addition, each millimeter of air-exposed dialysis probe resulted in an additional 0.4% evaporation of the dialysate. Therefore, in vitro dialysate glucose concentrations (including the above-mentioned results) were divided by a factor of 1.037. As the length of the air-exposed dialysis fiber was 1–2 mm on both the inlet and outlet connection, in vivo dialysate glucose concentrations were divided by a factor of 1.05.

In Vivo Experiments By use of a 23-gauge cannula, the dialysis probes were placed most superficially in the skin on the ventral part of the forearm 10 cm below the cubital region. The dialysis fiber was led through the cannula, the cannula was withdrawn, and the outlet tubing was positioned and glued. By gluing the outlet tubing after implantation, the size of the cannula could be reduced from 18 gauge to 23 gauge. The length of the dialysis fiber positioned in the skin was 20 mm. Two adjacent dialysis fibers were positioned 30 mm apart. In one subject, a single dialysis probe was implanted; in two subjects three probes were inserted. The subjects were investigated in the supine position and the measurements were performed after an overnight fast. In some subjects, EMLA cream

(Astra) and Tegaderm (3M) occlusion were applied 30 min before the implantation. Venous blood samples were drawn from the contralateral cubital vein.

In order to ensure the intracutaneous position of the dialysis probes, ultrasound scannings were performed. A 20-MHz scanner (Dermascan C, Cortex Technology, Denmark) was used. The ultrasound velocity was 1580 m/second.

Hyperemia caused by the probe implantation was measured by laser Doppler flowmetry (Pf1, Perimed, Sweden). Upper frequency limit was 12 kHz; time constant was 10 seconds.

In Vivo Calibration It has been shown that in vivo recovery does not correspond to in vitro recovery as mass transport in the tissues differs from that in aqueous solutions [3,7]. Lönnroth et al [3] presented an in vivo calibration technique that allows estimation of the exact in vivo recovery. The glucose concentration in the dialysate mirrors the recovery of the glucose gradient by the dialysis membrane. Because the recovery of glucose was constant over a wide range of glucose concentrations, a linear relationship is established in the dialysate and the concentration gradient over the dialysis membrane. If, then, the dialysis probe is perfused with different concentrations of glucose, a linear relationship is established between the net increase of the glucose concentration in the dialysate and the concentration of glucose in the inlet of the tubing. By using regression analysis, the concentration of glucose in the perfusate not resulting in any net influx of glucose can be calculated. At this glucose concentration, the gradient across the dialyzing membrane is zero; thus, the glucose concentration is in equilibrium with that of the surrounding tissue. Mean recovery of glucose is obtained from the slope of the regression line. The linear regression analysis was based on four to five different glucose concentrations (ranging from 0.6–7 mM glucose) using the least-squares method. The different glucose concentrations were used in a random order.

In two subjects, the experiments were initiated immediately after implantation of the probes. In four subjects, the experiments were not performed until the injection trauma had vanished, i.e., at least 2 h after implantation. In two subjects, the calibration was performed 18 h after implantation. One person was studied on two occasions (different probes were tested, during the implantation trauma phase and 18 h after implantation, respectively).

Glucose Analysis Glucose concentrations in dialysate and plasma were measured using the glucose oxidase method [8] on a Beckmann Glucose Analyzer (Beckman Instruments, Fullerton, CA). The glucose concentration in plasma water was calculated as 106% of the glucose concentration of the plasma glucose concentration, assuming the plasma protein concentration to be 60 g/l.

RESULTS

Without local analgesia, the probe implantation procedure caused a moderate, short-term discomfort. No pain was registered after application of EMLA cream.

The traumatic hypermia had vanished 90–135 min after implantation as measured by laser Doppler flowmetry (data not shown). In most subjects, the post-implantation baseline was similar to the pre-implantation baseline value.

C-mode ultrasound scanning of the probe position was performed on seven fibers, all of which were positioned in the dermis without detours into the underlying subcutaneous tissue. The scanning apparatus was unavailable during most of the experiments.

The in vivo calibration procedure based on perfusion of the dialysis probe with different concentrations of glucose was used to estimate in vivo recovery and absolute glucose concentrations in the skin. An example is presented in Fig 2.

The r value of the regression lines was 0.93 ± 0.03 (mean ± SEM). Three fibers had r values below 0.8 (range 0.65–0.74). If these fibers were not taken into consideration, mean r value was 0.97 ± 0.01.

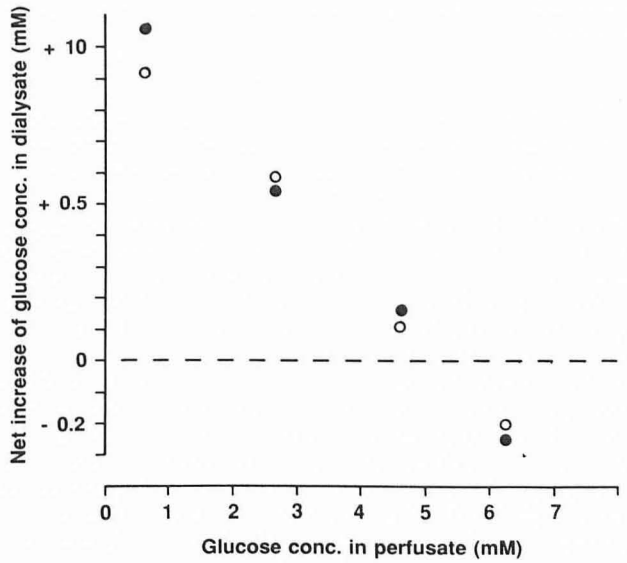

Figure 2. An in vivo calibration is shown. For further details, refer to *Materials and Methods.* In this example, data from two fibers (represented by *open* and *solid* circles, respectively) are shown. Both coefficients of correlation were 0.998. Recovery was 20.5 and 23.0%, respectively. The skin glucose level (5.3 and 5.2 mM) was identical with that in venous plasma water (5.3 mM). The experiment was performed after the implantation trauma had vanished.

During the implantation trauma phase, recovery was 32.3 ± 0.1%. After the trauma had vanished, recovery was 19.9 ± 1.3%, and 18 h after implantation recovery was 21.6 ± 0.6%. For the entire post-trauma period, recovery was 20.5 ± 0.7%. The coefficient of variation on adjacent fibers on recovery was 10.9%.

The skin glucose concentration was 99.1 ± 1.8% of the glucose concentration in venous plasma water. If only fibers with r values greater than 0.8 were taken into consideration, the skin/plasma glucose ratio was 98.3 ± 1.6%. The coefficient of variation on adjacent fibers on the estimation of the skin glucose concentration was 4.1%.

Control measurements were performed to investigate whether the decline in recovery from the time of implantation and afterwards was irreversible, for instance, caused by plugging the pores of the dialysis membrane with dermal proteins.

Six dialysis fibers were implanted in two fasting healthy subjects as described above. The experiments were performed 2–3 h after insertion of the fibers. A steady-state perfusion baseline was obtained for 20 min. Then the skin above each fiber was epicutaneously challenged with histamine chloride 16 mg/ml. Three prick tests were performed along each fiber in order to ensure that the entire fiber was covered by wheals. Glucose was collected in 30-μl (10 min) fractions. The results are shown in Table I. The dialysate glucose concentrations following skin-prick testing are presented relative to baseline values. The glucose concentration increased about 100% following histamine challenge. In all cases, the entire fiber was covered by wheals.

Table I. Changes in Dialysate Glucose Concentrations After Induction of Skin Inflammation[a]

Baseline	0–10 min	10–20 min	20–30 min	30–40 min
100%	146 ± 5%	194 ± 9%	174 ± 11%	165 ± 15%

[a] At time zero, the skin above each fiber was challenged with histamine. Values are mean ± SEM of six fibers implanted in two fasting subjects.

In all subjects, the entire fiber material was removed after the experiments.

DISCUSSION

The present study demonstrates for the first time the usefulness of the microdialysis technique for quantitative characterization of substances in the intercellular water space in intact human skin in vivo.

We found skin glucose levels identical to those in venous plasma water. However, we ignored the interstitial protein content, which probably is 7–25 g/l [10]. Thereby we may have underestimated the glucose concentration in the skin by 0.7–2.5%. Our results support the concept that glucose is not concentrated in the skin as previously considered ([9] for review).

The microdialysis technique opens up possibilities for evaluation of metabolic processes in intact human skin. In contrast to blister models, the epidermal-dermal interaction is preserved, the technique reflects in situ biochemical processes, and enzymatic degradation of the samples is minimized. However, several conditions must be taken into consideration. Drainage of substances from the tissue must be prevented or minimized, and the dialysis surface area prone to diffusion must be constant throughout the experiments. In other applications, it has been demonstrated that microdialysis probes can drain glucose [3], calcium [7], and other substances from the tissue. Recently, Hickner et al [11] showed that the glucose concentration in the dialysate increased and decreased parallel with changing muscle blood flow. The authors suggest that increased blood flow counteracts local drainage of glucose, and decreased blood flow expedites glucose drainage. Changes in blood flow per se appear to have no influence on recovery [12].

In our experiments, we used perfusates with glucose in order to avoid drainage. Our finding that the calibration technique estimated the skin glucose concentration to be similar to plasma glucose concentration, both during the implantation trauma period and after the trauma had vanished, suggest that no drainage of glucose took place in the skin in our experiments. Control measurements showed that recovery of glucose was constant for at least 2 h even without glucose added to the perfusate (unpublished observations). Therefore, drainage of glucose seems to be no problem in normal skin during short-term measurements.

Additionally, one must make sure that the dialysis capacity of the fiber is constant throughout the experiment. However, it was demonstrated that the recovery was variable. During the implantation trauma, recovery was about 50% greater than after the trauma had vanished. Control measurements with histamine skin-prick tests showed that the dialysate glucose concentration increased by 100% during the wheal reaction. Assuming that no concentration of glucose took place in the skin during the hyperemia, the increased dialysate glucose concentration must be caused by increased recovery of glucose. Consequently, we postulate that increased recovery during the implantation trauma phase was caused by trauma-induced vascular leakage, plasma extravasation, and edema formation, creating an increased probe surface area prone to diffusion. After the trauma had vanished, recovery was constant for at least 16 h.

In our set-up, 24–36 μl was needed to establish a new steady state of recovery after acute changes in glucose concentrations in the medium. This time resolution might limit the use of the microdialysis technique for some clinical research involving acute-phase reactions. However, the time resolution depends on the perfusion rate, the substances studied, and the analytical technique. We suppose that the microdialysis technique can be modified for use in many branches of dermatologic research.

EMLA cream was used to avoid discomfort during implantation. Control measurements performed 2 h after removal of the EMLA cream suggest that the analgetic effect had vanished at that time. Skin sensitivity to needles and histamine skin-prick test–induced flare reactions on the treated skin areas were comparable to values on untreated skin. The short-lasting action of EMLA cream in our application might be explained by an increased elimination rate of the analgetic substances due to skin hyperemia. In other types of experiments, further control studies are needed in order to exclude interference from the EMLA cream.

The in vivo calibration procedure is time consuming and is suitable only for steady-state conditions. On the other hand, semiquantitative microdialysis seems to possess a broad range of possibilities. As the coefficient of variation on recovery was acceptable, comparisons between dialysis probes are feasible without in vivo calibration.

On the basis of the present observations, we conclude that skin microdialysis might be a valuable tool in dermatologic research.

We thank Dr. Jørgen Serup for technical assistance with the ultrasound scannings.

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